Respiratory chain supercomplexes in the plant mitochondrial membrane

Dudkina, N.; Heinemeyer, J.; Sunderhaus, S.; Boekema, E.J.; Braun, H.P.

Published in:
Trends in Plant Science

DOI:
10.1016/j.tplants.2006.03.007

Publication date:
2006

Review TRENDS in Plant Science Vol.11 No.5 May 2006

Respiratory chain supercomplexes in the plant mitochondrial membrane

Natalya V. Dudkina[1,*], Jesco Heinemeyer[2,*], Stephanie Sunderhaus[2], Egbert J. Boekema[1] and Hans-Peter Braun[2]

[1]Department of Biophysical Chemistry, GBB, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
[2]Institute for Plant Genetics, Faculty of Natural Sciences, Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany

The intricate, heavily folded inner membrane of mitochondria houses the respiratory chain complexes. These complexes, together with the ATP synthase complex, are responsible for energy production, which is stored as ATP. The structure of the individual membrane-bound protein components has been well characterized. In particular, the use of Blue-native polyacrylamide gel electrophoresis has been instrumental in recent years in providing evidence that these components are organized into supercomplexes. Single particle electron microscopy studies have enabled a structural characterization of some of the mitochondrial supercomplexes. This has provided the opportunity to define a functional role for these supercomplexes for the first time, in particular for the dimeric ATP synthase complex, which appears to be responsible for the folding of the inner mitochondrial membrane.

Structure and function of the mitochondrial OXPHOS system

The mitochondrial oxidative phosphorylation (OXPHOS) system consists of four multi-subunit oxidoreductases involved in respiratory electron transport (Complexes I to IV) and the ATP synthase complex (Complex V). Except for Complex I, a considerable amount of information is known about the structure of the OXPHOS complexes of fungi and animals based on X-ray crystallography and biochemical investigations.

Complex I

Complex I (NADH–ubiquinone oxidoreductase) is the major entrance point of electrons to the respiratory chain [1]. It has a molecular mass of ~1 MDa and is composed of two elongated domains that together form an L-like structure. One domain is localized within the inner mitochondrial membrane and is involved in proton translocation; the other domain protrudes out of the plain of the membrane into the mitochondrial matrix and is responsible for oxidation of NADH. Approximately 40 different subunits are known to form part of Complex I [2,3].

Corresponding authors: Boekema, E.J. (e.j.boekema@rug.nl), Braun, H-P. (braun@genetik.uni-hannover.de).
* These authors contributed equally to this article.
Available online 17 April 2006

Complex II

Complex II (succinate–ubiquinone oxidoreductase) is a second entrance point of electrons to the respiratory chain [4,5]. It is the smallest complex of the OXPHOS system and consists of two soluble matrix-exposed subunits that are attached to two hydrophobic membrane proteins.

Complex III

Complex III (ubiquinol–cytochrome *c* oxidoreductase) represents the central component of the OXPHOS system [6,7]. It is a functional dimer of ~500 kDa composed of 2×10 or 11 distinct subunits. About a quarter of the complex is embedded within the inner mitochondrial membrane, a small part protrudes out into the mitochondrial intermembrane space and a larger part protrudes into the mitochondrial matrix.

Complex IV

Complex IV (cytochrome *c*–O_2 oxidoreductase) represents the terminal complex of the respiratory chain [8,9]: 12 to 13 subunits together form a monomer of ~220 kDa. It can exist as a monomer or a dimer within the membrane.

Complex V

The ATP synthase complex (Complex V) is a bipartite structure composed of a so-called F_1 headpiece within the mitochondrial matrix, which is anchored to a hydrophobic F_0-part within the inner mitochondrial membrane [10]. The two parts of Complex V are linked by a central stalk that rotates during catalysis and by a peripheral stalk that prevents rotation of the entire headpiece. The rotation of subunits within the two subcomplexes of Complex V is caused by the proton gradient across the inner mitochondrial membrane and forms the basis for phosphorylation of ADP. Complex V comprises ~15 distinct subunits, which partially are present in multiple copies within the holo-enzyme. The total molecular mass of Complex V is between 500 and 600 kDa.

Structure and function of the mitochondrial OXPHOS system in plants

The general structure and function of the plant OXPHOS complexes is considered to be closely related to those of the heterotrophic eukaryotes, although no particular structures have been analysed by X-ray crystallography. All five complexes include similar numbers of subunits, most

 1360-1385/$ - see front matter doi:10.1016/j.tplants.2006.03.007

of which are homologous to components of the corresponding yeast or bovine protein complexes [11]. However, some plant-specific subunits occur, which in some cases introduce side-activities into OXPHOS complexes. In all organisms, the acyl carrier protein of the mitochondrial fatty acid biosynthesis pathway forms part of Complex I [12,13]. In addition, in plants, L-galactono-1,4-lactone dehydrogenase (which represents the terminal enzyme of the mitochondrial ascorbic acid biosynthesis pathway) and carbonic anhydrases form part of Complex I [14,15]. Complex III includes the two subunits of the mitochondrial processing peptidase in plants, which is responsible for removing mitochondrial pre-sequences from nuclear-encoded mitochondrial proteins after transport has been completed [16]. Also, complexes II and IV include some additional plant-specific subunits that probably integrate extra functions into these OXPHOS complexes [17,18].

The functional context of mitochondrial respiration differs in autotrophic and heterotrophic organisms. In plants, mitochondria and plastids are involved in the redox balance of the cell [19]. Furthermore, mitochondria indirectly participate in photosynthesis through the 'photorespiration' pathway. Probably to accomplish these extra functions, additional oxidoreductases form part of the OXPHOS system in plants, such as the 'alternative oxidase' and three to four different 'rotenone-insensitive NAD(P)H dehydrogenases' [20–23]. All these enzymes participate in electron transport without contributing to the proton gradient across the inner mitochondrial membrane and therefore appear to catalyse wasteful reactions that are nevertheless considered to be of great importance under certain physiological conditions. Structurally, these enzymes do not form part of multi-enzyme complexes but instead exist as monomers or homo-dimers [22]. Unlike the classical OXPHOS enzyme complexes, which include nuclear and mitochondrially encoded subunits, these enzymes are all encoded by the nuclear genome.

Fluid state versus Solid state model of the OXPHOS system

The supramolecular organization of the OXPHOS system in mitochondria is a matter of debate. According to the 'Fluid-state' model, the five OXPHOS complexes independently diffuse within the inner mitochondrial membrane; electron transfer from one complex to another is based on random collisions between the complexes. By contrast, the 'Solid-state' model postulates stable interactions between the OXPHOS complexes under *in vivo* conditions. Experimental results supporting the Fluid-state model are based on the finding that all OXPHOS complexes can be biochemically purified in an enzymatically active form, and on diffusion rate measurements of OXPHOS complexes reconstituted into phospholipid vesicles (reviewed in [24]). The Solid-state model is supported by results obtained by reconstitution experiments [25,26], flux control experiments [27,28], and results concerning mutants with respect to subunits of individual OXPHOS complexes that specifically affect other OXPHOS complexes [29–31]. A useful strategy to investigate the supramolecular association of the OXPHOS proteins is based on mild solubilization of mitochondrial membranes using non-ionic detergents and separation of the solubilized protein complexes using Blue-native polyacrylamide gel electrophoresis (BN-PAGE) [32,33]. Using this strategy, defined supercomplexes could be described that have a $I+III_2$, III_2+IV_{1-2}, $I+III_2+IV_{1-4}$ and V_2 composition (Figure 1 and Table 1). Here we highlight recent studies of the plant OXPHOS mitochondrial system that used BN-PAGE and other biochemical procedures to investigate the supramolecular association of the OXPHOS proteins. Some of the respiratory supercomplexes discovered are extremely stable in plants, enabling their low-resolution structure to be defined by single particle electron microscopy (EM) for the first time [15,34,35].

Methodological strategies for characterizing mitochondrial supercomplexes in plants

BN-PAGE has proved to be a powerful procedure for characterizing mitochondrial supercomplexes. The method is based on solubilizing mitochondrial membranes with non-ionic detergents and incubating the generated protein fractions with Coomassie-blue, which introduces negative charge into proteins without denaturing them [36]. Protein complexes and supercomplexes are subsequently resolved on polyacrylamide gradient gels. Upon combining with SDS-PAGE as the second gel dimension, complexes are dissected into their subunits, which form vertical rows of spots on the resulting 2D gels (Figure 1a,c–h). Alternatively, first dimension BN-PAGE can be combined with a second BN-PAGE (BN/BN-PAGE), which is carried out in the presence of a different detergent. For example, protein solubilization and the first gel dimension are carried out in the presence of digitonin and the second gel dimension in the presence of dodecylmaltoside. All supercomplexes specifically destabilized by the conditions of the second gel dimension are (partially) dissected into protein complexes, which migrate beneath the diagonal line on the resulting 2D gels (Figure 1b). Both 2D gel systems enable the supramolecular association of proteins of the OXPHOS system and of other systems (e.g. the photosystem-supercomplexes of chloroplasts) to be investigated [37].

Supercomplexes of sufficient stability can be structurally analysed by single particle EM. For this approach, isolated mitochondria are treated with non-ionic detergents and supercomplexes are resolved by sucrose gradient ultracentrifugation. Selected fractions can be directly used for EM analyses and image processing [15,34,35].

Complex I and the $I+III_2$ supercomplex of plants

Until recently, our knowledge of the composition and configuration of plant Complex I was limited. The location of most the ~40 subunits within the L-shaped complex is still not known. However, some useful conclusions can be drawn from a comparison of the low-resolution structure of a series of Complex I molecules of animals and fungi obtained by EM (http://www.scripps.edu/biochem/CI/research.html). A structural scheme of the *Arabidopsis* Complex I is presented in Figure 2. The complex

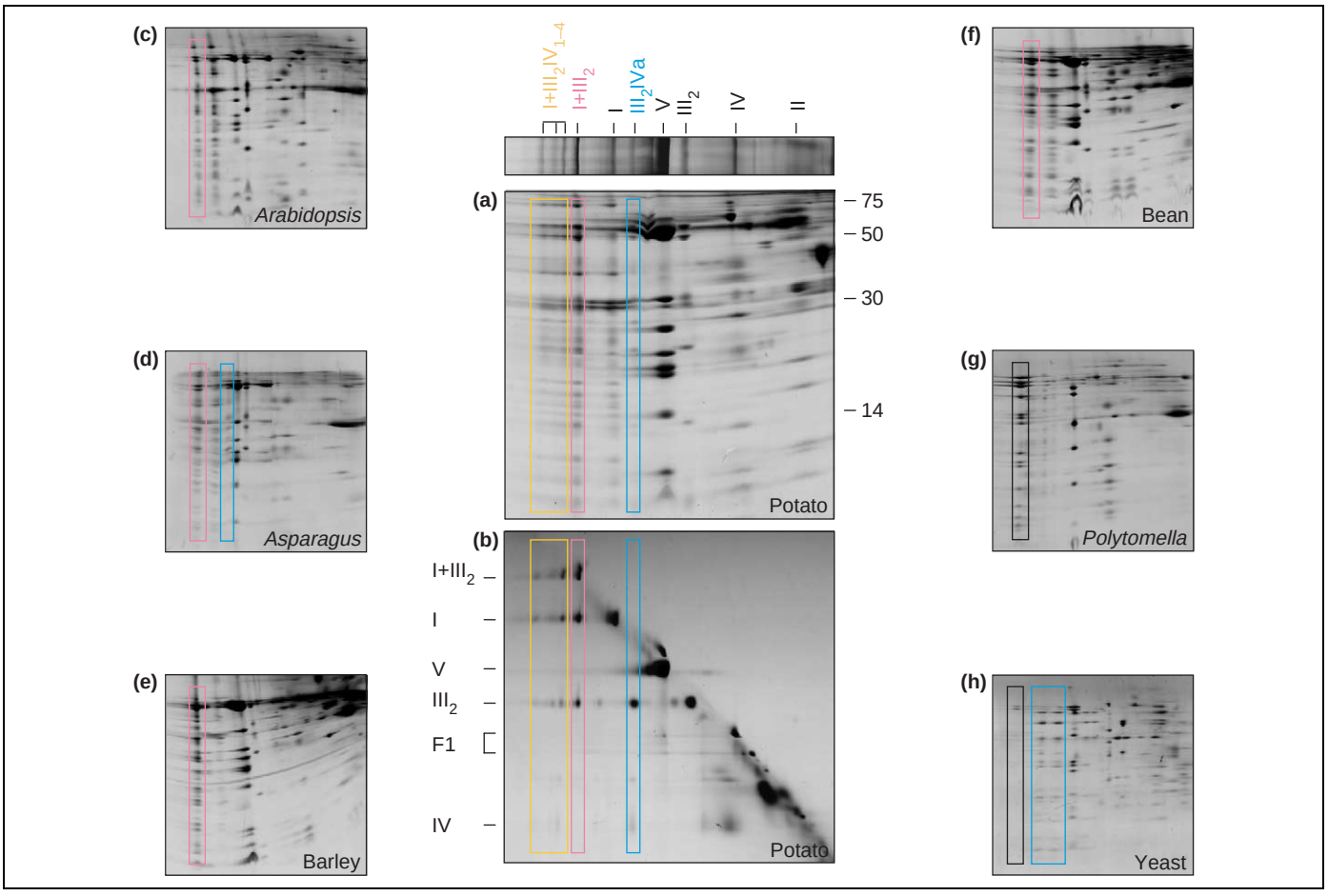

Figure 1. Separation of mitochondrial protein complexes by BN-PAGE. Mitochondrial fractions from potato, *Arabidopsis*, *Asparagus*, barley, bean, *Polytomella* and yeast were treated by digitonin (3 g detergent/g mitochondrial protein) and prepared for gel electrophoresis by addition of Coomassie Blue. Separation of mitochondrial protein complexes and supercomplexes from potato by **(a)** 2D BN/SDS-PAGE and **(b)** 2D BN/BN-PAGE (protocols according to [64] and [65]). Identity of protein complexes and supercomplexes of the OXPHOS system are indicated above the BN/SDS gel (a) and to the left of the BN/BN gel (b). Abbreviations: $I+III_2+IV_{1-4}$, supercomplex composed of Complex I, dimeric Complex III and 1–4 copies of Complex IV (boxed in yellow); $I+III_2$, supercomplex composed of Complex I and dimeric Complex III (boxed in pink); I, Complex I; V, ATP synthase complex; III_2, dimeric Complex III; III_2+IV, supercomplex composed of dimeric Complex III and Complex IV (boxed in blue); IV, Complex IV; II, Complex II. The molecular masses of standard proteins are given to the right of (a) (in kDa). Separation of mitochondrial protein complexes and supercomplexes from **(c)** *Arabidopsis*, **(d)** *Asparagus* (S. Sunderhaus, unpublished), **(e)** barley, **(f)** bean, **(g)** *Polytomella* and **(h)** yeast by BN/SDS-PAGE. Pink boxes indicate a supercomplex composed of Complex I and dimeric Complex III and blue boxes indicate a supercomplex composed of dimeric Complex III and Complex IV. Black boxes indicate a supercomplex composed of two ATP synthase complexes. (a) and (b) reproduced, with permission, from Ref. [44].

Table 1. OXPHOS supercomplexes in mitochondria identified by Blue-native PAGE[a]

Organism	V_2	$I+III_2$	III_2+IV_{1-2}	$I+III_2+IV_{1-4}$	Refs[b]
Arabidopsis	X	X			[17,49]
Barley		X			[17]
Bean		X			[17]
Potato		X[c]	X	X	[17,44]
Spinach	X	X	X	X	[48]
Tobacco		X			[66]
Pea		X			[67]
Sunflower				(X)[d]	[68]
Asparagus		X	X		[g]
Chlamydomonas	X				[55]
Polytomella	X	X			[35,56]
Saccharomyces cerevisiae	X	–[e]	X	–[e]	[32,33]
Podospora anserina	X	X[f]		X[f]	[69]
Bovine	X	X	X	X	[33]
Homo sapiens		X		X	[70]

[a]Empty cells in the table indicate that, to date, the corresponding supercomplexes have not been discovered, which could be due to low stability or their absence under *in vivo* conditions.
[b]Reference including the first report on the occurrence of a specific supercomplex.
[c]In potato two forms of $I+III$ supercomplexes occur, which have $I+III_2$ and I_2+III_4 composition.
[d]In sunflower, a complex IV containing supercomplex of >1000 kDa was described, which probably has $I+III_2+IV_{1-4}$ composition.
[e]The respiratory chain of *Saccharomyces cerevisiae* does not include complex I, therefore complex-I-containing supercomplexes are absent.
[f]In *Podospora anserine*, Complex I containing supercomplexes were reported to have I_2 and I_2III_2 composition.
[g]S. Sunderhaus, unpublished. See Figure 1d.

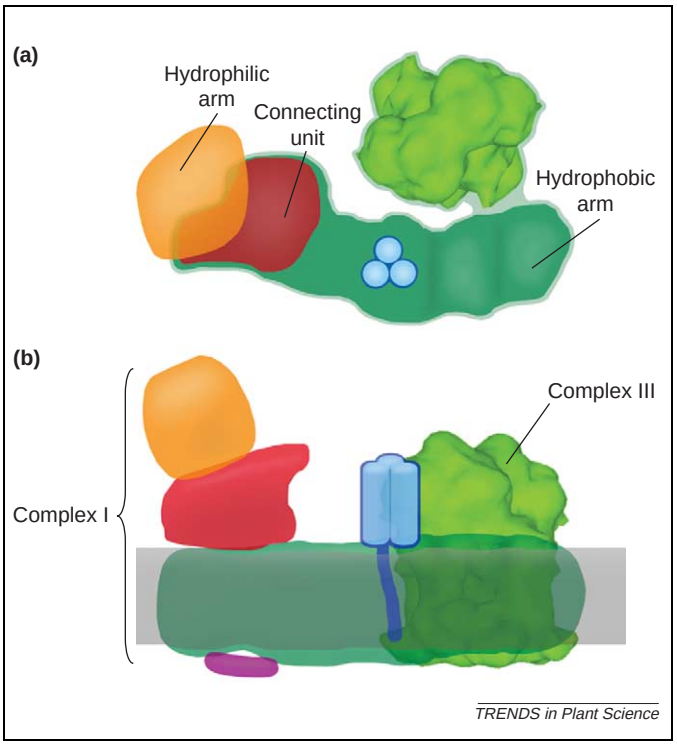

Figure 2. Structural scheme of the plant I+III_2 supercomplex within the membrane plane **(a)** and from the side **(b)**. The L-shaped Complex I comprises a membrane-bound hydrophobic arm (dark green) and a hydrophilic arm consisting of the NADH oxidizing unit (orange) and the connecting unit (red). These features have also been observed by electron microscopy in yeast and bovine mitochondria [1]. The NADH oxidizing unit is loosely attached in plants and algae but appears to be lacking in cyanobacteria [38]. A second hydrophilic protrusion (blue) is firmly fixed at the centre. It has been assigned to the heterotrimer of carbonic anhydrase and is present in the plant *Arabidopsis* and the alga *Polytomella* [15], but not in cyanobacteria [38]. An unknown third mass (not shown) is present at the tip of the hydrophobic arm in *Polytomella* [15] but not in *Arabidopsis*. A small intermembrane space-exposed protrusion (purple) is specific for *Arabidopsis* and *Polytomella* [15] but absent in Complex I particles from other organisms. Complex III (bright green) is connected to the membrane arm of Complex I mainly via its membrane-inserted portion.

is L-shaped, with a membrane-bound hydrophobic domain (dark green) and a matrix exposed hydrophilic domain (red and orange). The NADH oxidizing unit (orange) is loosely attached in *Arabidopsis* and in the alga *Polytomella* because breakdown products are often observed [15,34]. However, the NADH oxidizing unit appears to be absent in cyanobacteria [38]. A small intermembrane-space-exposed protrusion (purple) is specific for *Arabidopsis* and *Polytomella* [15,34] but absent in the Complex I particles of animals and fungi. Another striking special feature of the Complex I of plants and algae is the presence of a second matrix-exposed domain that is attached to the central part of the membrane arm (blue mass, Figure 2) [15,34]. Complex I from plants includes approximately ten plant-specific subunits [39,40], between three and five of which resemble a γ-type carbonic anhydrase of the archaebacterium *Methanosarcina thermophila* [41,42]. Analyses of Complex I subcomplexes of *Arabidopsis* using mass spectrometry and protease protection experiments suggest that the carbonic anhydrase subunits represent the second matrix-exposed domain of Complex I [15]. These subunits are probably present as a heterotrimer because the plant proteins have trimer-specific homologies to the archaebacterial protein. The carbonic anhydrase domain appears to be firmly attached to the membrane arm of Complex I because no particles without this protrusion were observed; this is probably achieved by membrane-inserted helical anchors close to the C-termini of the proteins. Possibly these carbonic anhydrases of Complex I are involved in an inner-cellular carbon transport system in higher plants that resembles the carbon concentration system of cyanobacteria [43]. Carbonic anhydrases also form part of the cyanobacterial Complex I. However, they do not form a similar second lumenal-exposed domain upon EM analysis (A.A. Arteni *et al.*, unpublished). An unknown third additional mass is present at the tip of the hydrophobic arm in *Polytomella* [15] but not in *Arabidopsis*.

On BN gels, Complex I forms part of a 1.5 MDa supercomplex that includes dimeric Complex III (Figure 1). This supercomplex is composed of all visible subunits of Complex I and Complex III and consequently is assumed to comprise at least 50 different types of polypeptides [17]. Using BN/BN-PAGE, the supercomplex becomes partially dissected into monomeric Complex I and dimeric Complex III (Figure 1b). No additional protein components form part of the I+III_2 supercomplex. In contrast to yeast and bovine mitochondria [33], the I+III_2 supercomplex of plant mitochondria is one of the dominant structures on BN gels, indicating high abundance or stability. More than 50% of Complex I is present within the I+III_2 supercomplex in potato and barley and ~50% of Complex I forms part of the supercomplex in *Arabidopsis*, *Asparagus*, bean and *Polytomella* [17,35,44]. Based on flux control experiments and BN-PAGE, supercomplexes and corresponding monomeric OXPHOS complexes are assumed to co-exist in mitochondria under *in vivo* conditions [28,44] and possibly assemble and disassemble in a dynamic manner. In potato, tissue-specific differences concerning supercomplex occurrence were observed [44]. Because of its high stability, the I+III_2 supercomplex of *Arabidopsis* was the first OXPHOS supercomplex to be characterized by single particle EM [34].

Computer modelling using the three-dimensional structure of bovine Complex III (reviewed in [5]) and the single particle EM structure of *Neurospora* and bovine Complex I [45,46] revealed that the interaction between both structures is within the membrane and that the matrix exposed hydrophilic parts of both complexes are not in close contact (Figure 2) [34]. Complex III is laterally attached to the membrane arm of Complex I, which is slightly bent around Complex III. The high stability of the I+III_2 supercomplex in plants might be due to the length of the membrane arm of Complex I, which is extended compared with the corresponding arm in animals and fungi (~230 Å versus ~190 Å). The physiological implications of the interaction between Complexes I and III_2 are not yet fully understood. The bovine I+III_2 supercomplex was shown to have higher NADH:cytochrome *c* oxidoreductase activity than the corresponding separate complexes under *in vitro* conditions [33]. This increase in activity can probably not be explained by direct ubiquinone channeling because the ubiquinone reduction site is believed to be located at the membrane arm close to its

interface with the matrix arm [1]. However, the physiology of the membrane arm of Complex I is largely unknown. It is speculated to include further proton (and other) translocation activities that might interact with the physiological processes of Complex III.

The III_2+IV and $I+III_2+IV_{1-4}$ supercomplexes of plants

Analyses of bovine mitochondria by BN-PAGE revealed abundant supercomplexes consisting of the OXPHOS complexes I, III_2 and IV [33]. Up to four copies of Complex IV are present within these supercomplexes. Corresponding particles were given the name 'respirasomes' because they can autonomously carry out respiration in the presence of the mobile electron carriers ubiquinone and cytochrome *c*. In mitochondria of *Saccharomyces cerevisiae*, which do not contain Complex I, stable III_2+IV_{1-2} supercomplexes were described by BN-PAGE [33,47]. These particles were also identified in bovine mitochondria but are of lower concentration [33]. In plants, supercomplexes containing Complexes III and IV are of low abundance on BN gels (Figure 1). Respirasomes were described for potato and spinach and a III_2+IV supercomplex for potato, spinach and *Asparagus* [44,48]. Hardly any Complex IV-containing supercomplexes were observed in *Arabidopsis* upon analysis by BN-PAGE and, to date, no single particle EM structures have been published of Complex IV-containing supercomplexes from any organism.

Dimeric ATP synthase supercomplex of plants

A dimeric ATP synthase supercomplex was first discovered for yeast mitochondria by BN/SDS-PAGE [32]. The supercomplex includes dimer-specific subunits termed e, g and k. More recently, a dimeric ATP synthase supercomplex was described for *Arabidopsis* on the basis of BN-PAGE [17,49]. This supercomplex is most stable upon solubilization of mitochondrial membranes using low Triton X-100 concentrations, which was previously reported for yeast [32]. However, compared with the $I+III_2$ supercomplex, dimeric ATP synthase is a fragile structure in higher plants. Disruption of the nuclear gene encoding the yeast subunit g led to the absence of dimers, indicating an important role for this protein in supercomplex assembly or stability. Ultrastructural studies on this yeast mutant also indicated that cristae were absent, which led to the suggestion that dimeric ATP synthase is essential for folding the inner mitochondrial membrane into cristae [50,51]. A similar prevention of cristae formation was described upon *in vivo* crosslinking of F_1 headpieces in yeast [52]. Previously, oligomeric ATP synthase complexes were identified by rapid-freeze deep-etch EM. These oligomers were proposed to be essential for folding the inner mitochondrial membrane [53,54]. However, until recently, precise information about the role of dimerizing ATP synthase was lacking.

A stable ATP synthase supercomplex was found in the algae *Chlamydomonas* and *Polytomella* [55,56] (Figure 1). This supercomplex could be purified by sucrose gradient ultracentrifugation and studied by single particle EM [35]. In these dimers, the monomers make an angle of ~70° with their long axes (Figure 3b). The kink in the lower part

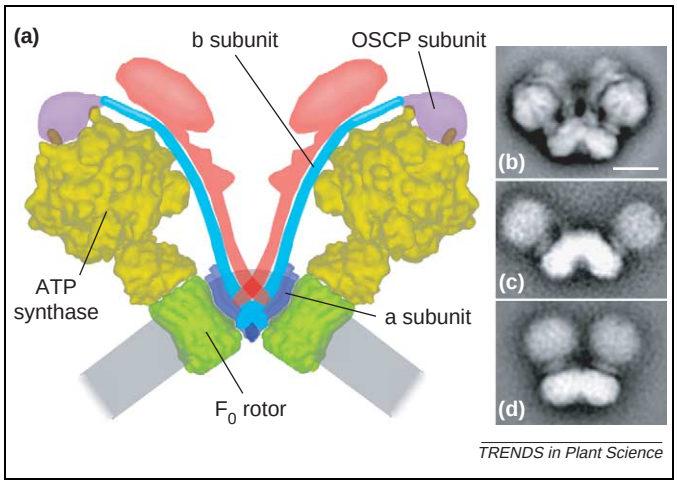

Figure 3. (a) Scheme for dimeric ATP synthase from mitochondria. Each ATP synthase monomer consists of the F_1 headpiece and a central stalk region (yellow) connected to the membrane-embedded F_0 rotor (green). Rotation of the complete headpiece is prevented by the b subunit of the peripheral stalk (blue), which is connected to the top of F_1 by the OSCP subunit (purple) and to the rotor by subunit a (dark blue). Other dimer-specific subunits that do not have direct functional importance for the monomers are depicted in red. **(b)** Projection map of *Polytomella* ATP synthase dimers [35]. Scale bar = 10 nm. **(c)** Projection map of *Saccharomyces cerevisiae* ATP synthase true-dimers (N.V. Dudkina *et al.*, unpublished). **(d)** Projection map of *S. cerevisiae* ATP synthase pseudo-dimers.

of the dimer causes a remarkable separation of the F_1 headpieces by more than 50 Å, preventing any direct contact between them. Hence, interaction of the monomers can only be realized by dimer-specific subunits within the membrane plane (Figure 3a, red). Interaction is probably also facilitated by the two peripheral stalks, which are facing each other. The ATP synthase supercomplex from *Polytomella* includes an additional 60 kDa protein termed 'Mitochondrial ATP synthase associated protein' or MASAP, which is supposed to be responsible for the high stability of the dimers. The MASAP subunit is probably part of the large mass in the upper half of the dimer (Figure 3a, red) close to the OSCP subunit (Figure 3a, purple), which links the b subunit of the peripheral stalk (Figure 3a, blue) to the F_1 headpiece. Such a large additional mass is lacking from analysed dimers of the yeast *S. cerevisiae* (Figure 3c). The yeast dimers were purified and analysed in a similar way to those of *Polytomella* (N.V. Dudkina *et al.*, unpublished) but show some distinct differences. Because of the lack of a large additional dimer-specific mass outside the membrane, tentatively assigned to the MASAP subunit, the peripheral stalks are thinner or hardly visible. However, the membrane-embedded F_0 parts are wider and kinked even more strongly, making an angle of ~90°. The wider diameter of the F_0 parts causes the F_1 headpieces to be separated even more strongly. It has been suggested that the yeast subunits 6, 8 (homologous to bovine A6L), b, f, g, i and k are present in this larger interface together with the peripheral stalk [50], but their exact location is not yet established. The precise homologues of some of these subunits in plants and *Polytomella*, if present, also need to be established. But, given the smaller membrane interface in *Polytomella*, it is likely that some of the yeast subunits do not have a counterpart.

In parallel to the ATP synthase dimer from *Polytomella*, the ATP synthase dimer of bovine mitochondria was analysed by EM [57]. This dimer has a configuration in which the headpieces are (almost) in contact, mainly because the angle between the monomers is only ~40°, which is strikingly different to the maps presented in Figure 3b,c. However, similar particles with an angle of 35° are also present in yeast (Figure 3d) (N.V. Dudkina *et al.*, unpublished). No intermediate angles were observed so it appears that both types of yeast particles could represent specific associates. The most logical explanation would be that these two dimers have a different composition. According to a scheme presented by Patrick Paumard *et al.* [50], the dimers arrange into linear oligomers in the membrane. We hypothesize that detergent solubilization of the oligomers could lead to 'true (native) dimers' as depicted in Figure 3b,c and to 'pseudo-dimers' consisting of two close-neighbour monomers from two different broken native dimers, as shown in Figure 3d. If correct, this interpretation would explain why the width of the F_0 moiety in the pseudo-dimer (Figure 3d) and in the bovine dimer [57] is much smaller than it is in the yeast true dimer (Figure 3c).

There must be a special reason for the occurrence of ATP synthase dimers in mitochondria because the monomer is perfectly designed for catalysing the synthesis of ATP, including mechanisms to regulate its activity. The shape of the *Polytomella*, bovine and yeast ATP synthase super-complexes gives a clue as to the role of dimerization. The unique orientation of the out-of-plane association of the F_0 membrane domains (Figure 3) will force a strong local curvature of the membrane [35,57]. Most of the ATP synthase complexes are not part of a flat inner mitochondrial membrane but occur within strongly curved invaginations known as cristae lamellae and tubules. For tubular membranes, the diameter is often in the range of 30 nm [58]. If the bent membrane in the region of the dimers is regarded as an arc section of radius 15 nm, this configuration could by extrapolation induce a tubule with a diameter of ~30 nm. Such a diameter would fit the observed cristae dimensions. It is likely that the ATP synthase dimers associate into specific oligomers and that the other respiratory chain supercomplexes are arranged between the ATP synthase oligomers. Indeed, oligomeric ATP synthase rows were previously described by rapid-freeze deep-etch EM [53,54]. We propose that ATP synthase dimers are the building blocks of ATP synthase oligomers, which are helically arranged in tubular cristae, as originally proposed by Richard Allen *et al.* [53]. The formation of these helical structures is the driving force for cristae formation and overall mitochondrial morphology as shown in Figure 4.

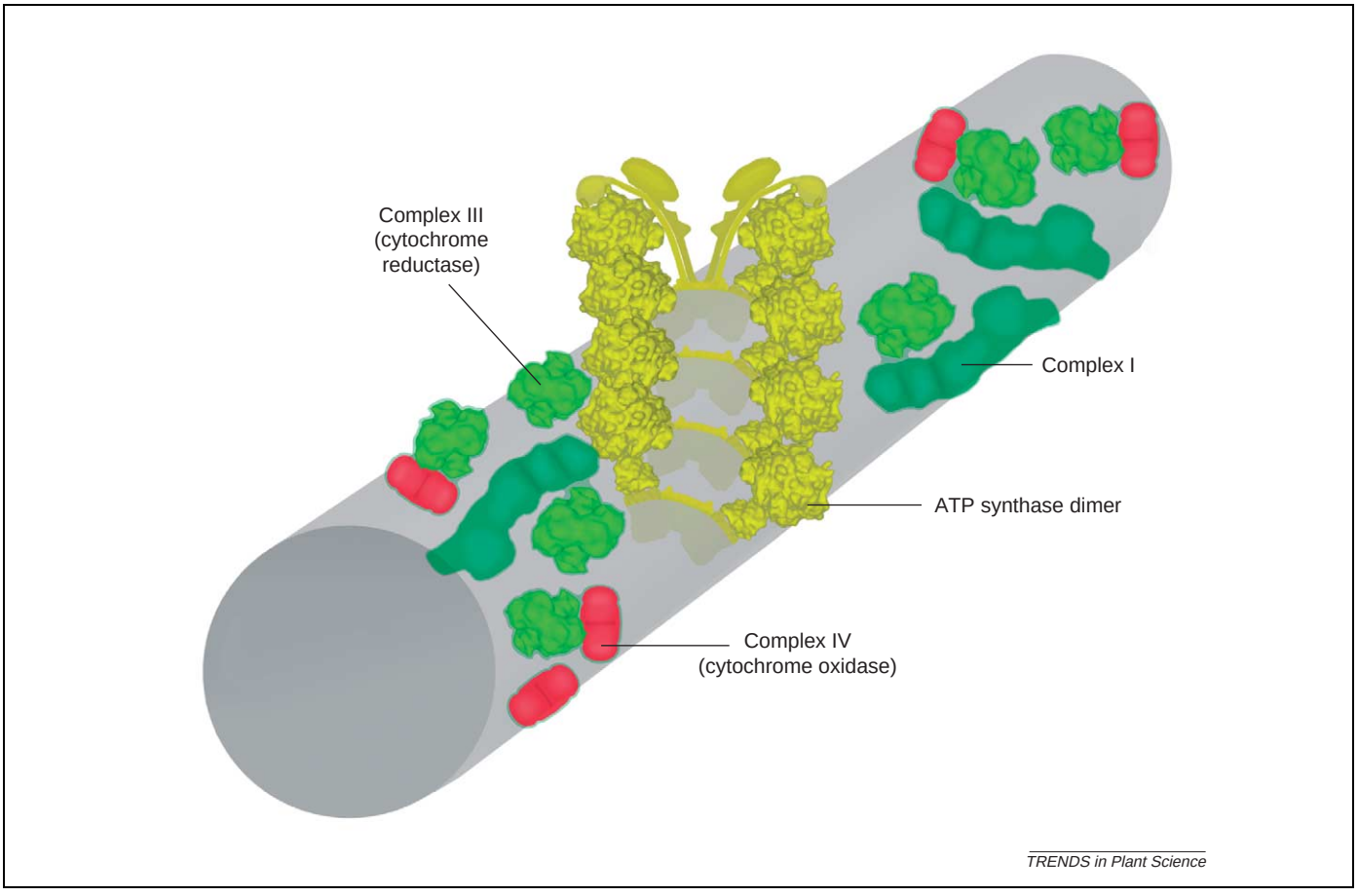

Figure 4. Proposed scheme for mitochondrial supercomplex packing within a tubular cristae membrane. The ATP synthase dimers (yellow) are arranged in row-like oligomers in a helical fashion, of which four are shown. The oligomers are thought to induce membrane curvature to form cristae rods. The three other large respiratory chain complexes are mostly organized as supercomplexes and are arranged in between the rows of ATP synthase. Dimeric Complex III (cytochrome reductase, bright green), monomeric Complex I (dark green) and dimeric Complex IV (cytochrome oxidase, red) are depicted here to form several types of supercomplexes. The exact interaction between Complex III and Complex IV has not yet been established and it could be possible that Complex III associates with two cytochrome oxidase monomers. The much smaller Complex II has no structural association with the other respiratory chain (Complexes III and IV) and has been omitted.

In this model, the other types of supercomplexes, composed of Complexes I, III and IV, are arranged in densely packed arrays between the rows of ATP synthase dimers. Wider or flattened tubules also appear to occur so there might be some variation on this theme and variation between species given that the dimers of *Polytomella* and yeast differ in shape (Figure 4).

A further question is whether the ATP synthase supercomplex arrangement (Figures 3 and 4) is unique to mitochondria. Based on BN-gel electrophoresis studies, the ATP synthase from *Chlamydomonas* chloroplasts is claimed to be dimeric as well [59]. However, an EM study of spinach chloroplasts indicated that the F_1 headpieces do not have any specific interaction within the membrane [60]. Because the chloroplast membranes are flat in the parts where the ATP synthase is located [60], there is no obvious reason why there should be (kinked) dimers in the chloroplast membranes. Hence, it can be concluded that the dimers are probably unique to mitochondria and that their interaction is primarily essential to enlarge the surface of the inner mitochondrial membrane by inducing its heavy folding.

Perspectives

The proposed organization of mitochondrial membranes rules out the possibility that this membrane is organized according to the 'Fluid-state' model. However, mitochondria and their membranes are regarded as flexible structures that can rapidly adapt in response to changing physiological requirements. Consequently, the OXPHOS system cannot be described by the static 'Solid-state' model. Single OXPHOS complexes and their supercomplexes probably dynamically co-exist within the inner mitochondrial membrane (Figure 4): this idea is supported by results obtained by BN-PAGE and by flux control measurements [28]. Furthermore, the stoichiometry of different OXPHOS complexes within the inner mitochondrial membrane differs, excluding the possibility that all complexes form part of a supercomplex at a given time. Cardiolipin is reported to play an important role in supercomplex formation in yeast mitochondria [61,62].

The physiological roles of OXPHOS supercomplexes have not yet been determined. *In vitro* activity measurements indicate that they form the basis for enhanced electron transfer rates between the complexes of the respiratory chain [33]. Furthermore, supercomplex formation has implications for the structural organization of the inner mitochondrial membrane. The morphology of the folds of the inner mitochondrial membrane varies in different organisms and, therefore, abundance and composition of specific respiratory supercomplexes can be expected to differ, which is supported by the results obtained by BN-PAGE (Figure 1). Based on classical thin sectioning it appears that three types of inner membrane folds can be distinguished: lamellar cristae, vesicular cristae and tubular cristae. The formation of ATP synthase dimers and oligomers is likely to be particularly important for tubular cristae, which is in line with results obtained by transmission EM for *Paramecium* and *Polytomella* [53] (N.V. Dudkina *et al.*, unpublished). The inner membrane folds of higher plants are thought to be more of the lamellar cristae type, which perhaps explains the comparatively weak interaction of ATP synthase monomers in this group of organisms. However, this is speculative and, moreover, classical techniques such as thin sectioning might give a rather distorted view of membrane morphology [58]. Higher-resolution EM tomography investigations [63] need to be performed on intact mitochondria to better understand correlations between the folding types of the inner mitochondrial membrane and the supercomplex composition within in this membrane.

Many other hypotheses concerning supercomplex function have been proposed [33,34]. Supercomplexes possibly allow reciprocal stabilization of OXPHOS complexes, they might offer efficient regulation of the respiratory chain or they could simply be important for increasing the amount of protein that can be inserted into the inner mitochondrial membrane. $I+III_2$ supercomplex formation was thought to regulate alternative respiration in plants because it possibly limits the access of the alternative oxidase to its substrate ubiquinol. The alternative oxidoreductases of plant mitochondria do not appear to form part of any of the respiratory supercomplexes described [17] and to date the regulation of these enzymes is not well understood. More precise information on the supramolecular organization of the OXPHOS system must await the structural characterization of further supercomplexes, particularly those that include Complex IV, the terminal respiratory chain oxidoreductase.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Dutch science foundation NWO-CW.

References

1 Friedrich, T. and Böttcher, B. (2004) The gross structure of the respiratory Complex I: a Lego system. *Biochim. Biophys. Acta* 1608, 1–9
2 Carroll, J. *et al*. (2003) Analysis of the subunit composition of Complex I from bovine heart mitochondria. *Mol. Cell. Proteomics* 2, 117–126
3 Abdrakhmanova, A. *et al*. (2004) Subunit composition of mitochondrial Complex I from the yeast *Yarrowia lipolytica*. *Biochim. Biophys. Acta* 1658, 148–156
4 Yankovskaya, V. *et al*. (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 299, 700–704
5 Horsefield, R. *et al*. (2004) Complex II from a structural perspective. *Curr. Protein Pept. Sci.* 5, 107–118
6 Berry, E.A. *et al*. (2000) Structure and function of cytochrome *bc* complexes. *Annu. Rev. Biochem.* 69, 1005–1075
7 Hunte, C. *et al*. (2000) Structure at 2.3 Å resolution of the cytochrome *bc*1 complex. from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody fragment. *Structure* 8, 669–684
8 Tsukihara, T. *et al*. (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. *Science* 272, 1136–1144
9 Michel, H. *et al*. (1998) Cytochrome c oxidase: structure and spectroscopy. *Annu. Rev. Biophys. Biomol. Struct.* 27, 329–356
10 Stock, D. *et al*. (2000) Rotary mechanism of ATP synthase. *Curr. Opin. Struct. Biol.* 10, 672–679
11 Vedel, F. *et al*. (1999) The mitochondrial respiratory chain and ATP synthase complexes: composition, structure and mutational studies. *Plant Physiol. Biochem.* 37, 629–643
12 Runswick, M.J. *et al*. (1991) Presence of an acyl carrier protein in NADH:ubiquinone oxidoreductase from bovine heart mitochondria. *FEBS Lett.* 286, 121–124

13 Sackmann, U. *et al*. (1991) The acyl-carrier protein in *Neurospora crassa* mitochondria is a subunit of NADH: ubiquinone reductase (Complex I). *Eur. J. Biochem.* 200, 463–469
14 Millar, A.H. *et al*. (2003) Control of ascorbate synthesis by respiration and its implications for stress responses. *Plant Physiol.* 133, 443–447
15 Sunderhaus, S. *et al*. (2006) Carbonic anhydrase subunits form a matrix-exposed domain attached to the membrane arm of mitochondrial Complex I in plants. *J. Biol. Chem.* 281, 6482–6488
16 Braun, H.P. *et al*. (1992) The general mitochondrial processing peptidase from potato is an integral part of cytochrome c reductase of the respiratory chain. *EMBO J.* 11, 3219–3227
17 Eubel, H. *et al*. (2003) New insights into the respiratory chain of plant mitochondria: supercomplexes and a unique composition of Complex II. *Plant Physiol.* 133, 274–286
18 Millar, A.H. *et al*. (2004) Mitochondrial cytochrome c oxidase and succinate dehydrogenase contain plant-specific subunits. *Plant Mol. Biol.* 56, 77–89
19 Dutilleul, C. *et al*. (2003) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signalling and diurnal regulation. *Plant Cell* 15, 1212–1226
20 Siedow, J.N. and Umbach, A.L. (1995) Plant mitochondrial electron transfer and molecular biology. *Plant Cell* 7, 821–831
21 Moller, I.M. (2002) A new dawn for plant mitochondrial NAD(P)H dehydrogenases. *Trends Plant Sci.* 7, 235–237
22 Juszczuk, I.M. and Rychter, A.M. (2003) Alternative oxidase in higher plants. *Acta Biochim Pol.* 50, 1257–1271
23 Rasmusson, A.G. *et al*. (2004) Alternative NAD(P)H dehydrogenases of plant mitochondria. *Annu. Rev. Plant Biol.* 55, 23–39
24 Hackenbrock, C.R. *et al*. (1986) The random collision model and a critical assessment of the diffusion and collision in mitochondrial electron transport. *J. Bioenerg. Biomembr.* 18, 331–368
25 Fowler, L.R. and Hatefi, Y. (1961) Reconstitution of the electron transport system III. Reconstitution of DPNH oxidase, succinic oxidase, and DPNH succinic oxidase. *Biochem. Biophys. Res. Commun.* 5, 203–208
26 Fowler, L.R. and Richardson, H.S. (1963) Studies on the electron transfer system. *J. Biol. Chem.* 238, 456–463
27 Boumans, H. *et al*. (1998) The respiratory chain in yeast behaves as a single functional unit. *J. Biol. Chem.* 273, 4872–4877
28 Bianchi, C. *et al*. (2004) The mitochondrial respiratory chain is partially organized in a supercomplex assembly: kinetic evidence using flux control analysis. *J. Biol. Chem.* 279, 36562–36569
29 Grad, L.I. and Lemire, B.D. (2004) Mitochondrial Complex I mutations in *Caenorhabditis elegans* produce cytochrome c oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis. *Hum. Mol. Genet.* 13, 303–314
30 Acin-Perez, R. *et al*. (2004) Respiratory Complex III is required to maintain Complex I in mammalian mitochondria. *Mol. Cell* 13, 805–815
31 Ugalde, C. *et al*. (2004) Differences in assembly or stability of Complex I and other mitochondrial OXPHOS complexes in inherited Complex I deficiency. *Hum. Mol. Genet.* 13, 659–667
32 Arnold, I. *et al*. (1998) Yeast mitochondrial F_1F_0-ATP synthase exists as a dimer: identification of three dimer-specific subunits. *EMBO J.* 17, 7170–7178
33 Schägger, H. and Pfeiffer, K. (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J.* 19, 1777–1783
34 Dudkina, N.V. *et al*. (2005) Structure of a mitochondrial supercomplex formed by respiratory chain Complexes I and III. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3225–3229
35 Dudkina, N.V. *et al*. (2005) Structure of dimeric ATP synthase from mitochondria: an angular association of monomers induces the strong curvature of the inner membrane. *FEBS Lett.* 579, 5769–5772
36 Schägger, H. and von Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal. Biochem.* 199, 223–231
37 Heinemeyer, J. *et al*. (2004) Proteomic approach to characterize the supramolecular organization of photosystems in higher plants. *Phytochemistry* 65, 1683–1692
38 Arteni, A.A. *et al*. (2005) Single particle electron microscopy in combination with mass spectrometry to investigate novel complexes of membrane proteins. *J. Struct. Biol.* 149, 325–331
39 Heazlewood, J.L. *et al*. (2003) Mitochondrial Complex I from *Arabidopsis* and rice: orthologs of mammalian and yeast components coupled to plant-specific subunits. *Biochim. Biophys. Acta* 1604, 159–169
40 Cardol, P. *et al*. (2004) Higher plant-like subunit composition of mitochondrial Complex I from *Chlamydomonas reinhardtii*: 31 conserved components among eukaryotes. *Biochim. Biophys. Acta* 1658, 212–224
41 Parisi, G. *et al*. (2004) Gamma carbonic anhydrases in plant mitochondria. *Plant Mol. Biol.* 55, 193–207
42 Perales, M. *et al*. (2005) Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces Complex I and supercomplex I+III2 levels and alters mitochondrial physiology in *Arabidopsis*. *J. Mol. Biol.* 350, 263–277
43 Badger, M.R. and Price, G.D. (2003) CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *J. Exp. Bot.* 54, 609–622
44 Eubel, H. *et al*. (2004) Identification and characterization of respirasomes in potato mitochondria. *Plant Physiol.* 134, 1450–1459
45 Guénebaut, V. *et al*. (1997) Three-dimensional structure of NADH-dehydrogenase from *Neurospora crassa* by electron microscopy and conical tilt reconstruction. *J. Mol. Biol.* 265, 409–418
46 Grigorieff, N. (1998) Three-dimensional structure of bovine NADH:ubiquinone oxidoreductase (Complex I) at 22 Å in ice. *J. Mol. Biol.* 277, 1033–1046
47 Cruciat, C.M. *et al*. (2000) The cytochrome bc_1 and cytochrome c oxidase complexes associate to form a single supracomplex in yeast mitochondria. *J. Biol. Chem.* 275, 18093–18098
48 Krause, F. *et al*. (2004) "Respirasome"-like supercomplexes in green leaf mitochondria of spinach. *J. Biol. Chem.* 279, 48369–48375
49 Eubel, H. *et al*. (2004) Respiratory chain supercomplexes in plant mitochondria. *Plant Physiol. Biochem.* 42, 937–942
50 Paumard, P. *et al*. (2002) The ATP synthase is involved in generating mitochondrial cristae morphology. *EMBO J.* 21, 221–230
51 Giraud, M.F. *et al*. (2002) Is there a relationship between the supramolecular organization of the mitochondrial ATP synthase and the formation of cristae? *Biochim. Biophys. Acta* 1555, 174–180
52 Gavin, P.D. *et al*. (2004) Cross-linking ATP synthase complexes *in vivo* eliminates mitochondrial cristae. *J. Cell Sci.* 117, 2333–2343
53 Allen, R.D. *et al*. (1989) An investigation of mitochondrial inner membranes by rapid-freeze deep-etch techniques. *J. Cell Biol.* 108, 2233–2240
54 Allen, R.D. (1995) Membrane tubulation and proton pumps. *Protoplasma* 189, 1–8
55 van Lis, R. *et al*. (2003) Identification of novel mitochondrial protein components of *Chlamydomonas reinhardtii*. A proteomic approach. *Plant Physiol.* 132, 318–330
56 Atteia, A. *et al*. (2003) Bifunctional aldehyde/alcohol dehydrogenase (ADHE) in chlorophyte algal mitochondria. *Plant Mol. Biol.* 53, 175–188
57 Minauro-Sanmiguel, F. *et al*. (2005) Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 102, 12356–12358
58 Frey, T.G. and Mannella, C.A. (2000) The structure of mitochondria. *Trends Biochem. Sci.* 25, 319–324
59 Rexroth, S. *et al*. (2004) Dimeric H^+-ATP synthase in the chloroplast of *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* 1658, 202–211
60 Dekker, J.P. and Boekema, E.J. (2005) Supermolecular organization of the thylakoid membrane proteins in green plants. *Biochim. Biophys. Acta* 1706, 12–39
61 Zhang, M. *et al*. (2002) Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. *J. Biol. Chem.* 277, 43553–43556
62 Pfeiffer, K. *et al*. (2003) Cardiolipin stabilizes respiratory chain supercomplexes. *J. Biol. Chem.* 278, 52873–52880
63 Medalia, O. *et al*. (2002) Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. *Science* 298, 1209–1213

64 Heinemeyer, J. *et al*. Blue-native gel electrophoresis for the characterization of protein complexes in plants. In *Plant Proteomics* (*Methods in Molecular Biology Series*) (Thiellement, H., ed.), Humana Press (in press)
65 Sunderhaus, S. *et al*. Two dimensional blue native/blue native polyacrylamide gel electrophoresis for the characterization of mitochondrial protein complexes and supercomplexes. In *Mitochondrial Genomics and Proteomics Protocols* (*Methods in Molecular Biology Series*) (Leister, D and Herrmann, J.H., eds), Humana Press (in press)
66 Pineau, B. *et al*. (2005) Targeting the NAD7 subunit to mitochondria restores a functional Complex I and a wild type phenotype in the *Nicotiana sylvestris* CMS II mutant lacking nad7. *J. Biol. Chem.* 280, 25994–26001
67 Taylor, N.L. *et al*. (2005) Differential impact of environmental stresses on the pea mitochondrial proteome. *Mol. Cell. Proteomics* 4, 1122–1133
68 Sabar, M. *et al*. (2005) Histochemical staining and quantification of plant mitochondrial respiratory chain complexes using blue-native polyacrylamide gel electrophoresis. *Plant J*. 44, 893–901
69 Krause, F. *et al*. (2004) Supramolecular organization of cytochrome c oxidase- and alternative oxidase-dependent respiratory chains in the filamentous fungus *Podospora anserina*. *J. Biol. Chem*. 279, 26453–26461
70 Schägger, H. *et al*. (2004) Significance of respirasomes for the assembly/stability of human respiratory chain complex I. *J. Biol. Chem.* 279, 36349–36353

Plant Science meetings in July 2006

Plant Growth Regulation Society of America (PGRSA) 33rd Annual Conference
9–12 July 2006
Quebec City Hilton, Quebec City, Canada
http://www.griffin.peachnet.edu/pgrsa

17th International Symposium on Plant Lipids
16–21 July 2006
East Lansing, MI, USA
http://www.ispl2006.msu.edu/index.html

XV FESPB 2006: Plants, People, Ecosystems and Applications
17–21 July 2006
Lyon, France
http://www.fespb2006.org/

5th International Conference on Mycorrhiza
23–27 July 2006
Granada, Spain
http://www.eez.csic.es/icom5/

Botany 2006
28 July – 3 August 2006
Chico, CA, USA
http://www.2006.botanyconference.org/

American Phytopathological Society
29 July – 2 August 2006
Québec, Canada
http://meeting.apsnet.org/